Soil C extracted with water or K_2SO_4: pH effect on determination of microbial biomass

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Haney, R. L., Franzluebbers, A. J., Hons, F. M. and Zuberer, D. A. 1999. **Soil C extracted with water or K_2SO_4: pH effect on determination of microbial biomass.** Can. J. Soil Sci. **79**: 529–533. Routine determination of soil microbial biomass C has shifted during the past decade from chloroform fumigation-incubation to chloroform fumigation-extraction using 0.5 *M* K_2SO_4 as extractant. We compared extractable C with water and 0.5 *M* K_2SO_4 in eight soils ranging in pH from 5.4 to 8.3. In unfumigated soils with low pH, extractable C was 0.8- to 1.2-fold greater with water than with 0.5 *M* K_2SO_4. However, in unfumigated soils with pH > 7.7, extractable C, although not statistically significant, was 11 to 19% less with water than with 0.5 *M* K_2SO_4. In fumigated soils, no difference in extractable C between water and 0.5 *M* K_2SO_4 was detected among soils with pH < 7.7, but extractable C was 13 to 17% less with water than with 0.5 *M* K_2SO_4 with pH > 7.7. Our results suggest that 0.5 *M* K_2SO_4 (1) may flocculate soil and cause adsorption of solubilized C onto colloids at pH < 7.7, but (2) may disperse calcareous soils at pH > 7.7, thereby differentially affecting the fate of solubilized C depending upon soil pH. Our results put into question the widespread adaptability of using chloroform fumigation-extraction to estimate microbial biomass C.

Key words: Extractable carbon, chloroform fumigation-extraction, microbial biomass

Haney, R. L., Franzluebbers, A. J., Hons, F. M. et Zuberer, D. A. 1999. **Extraction du C du sol dans l'eau ou dans K_2SO_4: effet du pH sur la détermination de la biomasse microbienne.** Can. J. Soil Sci. **79**: 529–533. Les méthodes régulières de mesure du C relié à la biomasse microbienne du sol sont passées, au cours de la dernière décennie, de la fumigation au chloroforme-incubation à la fumigation au chloroforme avec extraction, utilisant une solution 0,5 M de K_2SO_4 comme extractant. Nous avons comparé l'extraction du C à l'eau et dans 0,5 M K_2SO_4 dans 8 sols de pH allant de 5,4 à 8,3. Dans les sols à pH bas non fumigés, le C extractible était 0,8 à 1,2 fois plus abondant avec la méthode d'extraction à l'eau qu'avec celle à 0,5 M K_2SO_4. En revanche, dans les sols non fumigés à pH supérieur à 7,7, le C extractible, encore que non significatif au plan statistique, était de 11 à 19 fois moins abondant avec la méthode à l'eau. Dans les sols fumigés, aucune différence entre les deux méthodes n'était décelée quand le pH des sols était inférieur à 7,7 mais dans les sols à pH supérieur à 7,7, le C extractible était de 13 à 17 fois moins abondant avec l'extraction à l'eau. Il se dégage de nos observations (1) que 0,5 M K_2SO_4 peut floculer le sol et provoquer l'adsorption du C solubilisé sur les colloïdes à pH < 7,7, mais (2) qu'il disperse les sols calcaires à pH > à 7,7, déterminant du fait même le devenir différent du C solubilisé selon le pH du sol. Nos résultats viennent remettre en question l'efficacité de l'utilisation généralisée de la méthode de la fumigation au chloroforme suivie d'extraction pour estimer le C de la biomasse microbienne.

Mots clés: Carbone extractible, fumigation au chloroforme avec extraction, biomasse microbienne

Soil microbial biomass C is a useful indicator of soil quality and fertility (Gregorich et al. 1994). No standard protocol for measuring microbial biomass exists, but several widely differing approaches are employed, including chloroform fumigation-incubation, chloroform fumigation-extraction, adenosine triphosphate, and substrate-induced respiration. Strength of relationships among these various methods is excellent within a few soils from the same geographic region (Vance et al. 1987a; Ocio and Brookes 1990), generally adequate for ranking soils from the same geographical region (Kaiser et al. 1992; Sparling and Zhu 1993; Wardle and Ghani 1995; Anderson and Joergensen 1997; Beck et al. 1997), but poor among soils with widely differing organic matter contents from different regions (Horwath et al. 1996; Franzluebbers et al. 1999). Limitations in various methods have been evaluated in detail (Martens 1995).

Although chloroform fumigation-incubation has been the most commonly used method, and has been the standard by which other newer methods were compared, chloroform fumigation-extraction with 0.5 *M* K_2SO_4 has dominated recent research concerned with soil microbial biomass C (Wardle 1998). One reason for this shift has been the perceived ease and rapidity of obtaining microbial biomass estimates with chloroform fumigation-extraction compared with the 10-d incubation required with chloroform fumigation-incubation.

Chloroform does not apparently render all cell components soluble, leaving some microbial components unextracted, requiring an extraction efficiency factor (k_{EC}) to compensate for this unextracted C of microbial origin. Extraction efficiencies have been summarized among studies as 0.43 ± 0.10 (n = 74) (Martens 1995). Two-thirds of the k_{EC} values, therefore, lie within a range of 0.33 to 0.53, while 90% lie within the range of 0.23 to 0.63. Reasons for differing k_{EC} among soils, derived typically by extracting ^{14}C-labelled microorganisms, have not been adequately

explained. Although clays are thought to adsorb solubilized cell components following fumigation, available data do not support a strong relationship between k_{EC} and clay content (Kaiser et al. 1992). Other physico-chemical soil properties such as porosity, water-holding capacity, sand, silt, organic C, and carbonate content have been suggested as important variables controlling k_{EC} (Badalucco et al. 1997). Results from Anderson and Joergensen (1997) suggest that k_{EC} should be higher in acid than in neutral soils when compared with substrate-induced respiration, but suggest that k_{EC} should be lower in acid than in neutral soils when compared with basal soil respiration.

Chloroform fumigation-extraction uses 0.5 *M* K_2SO_4 as an extractant, although K^+ is known to flocculate soil colloids (Oades 1989). Flocculation can remove extractable C from solution when the flocculant causes clay interlayers and the diffuse double layer around colloids to collapse and previously soluble organic C binds to clay or organo-clay complexes. This effect is similar to that of "salting out" of proteins, which has been historically used in biochemistry research to separate proteins from other constituents.

Soluble organic C was extracted earlier with KCl (Tinsley 1950). Later, it was observed that Cl^- interfered with the colorimetric determination of soluble organic C using the then-popular potassium dichromate method (Quinn and Salomon 1964). Following that time, 0.5 *M* K_2SO_4 has been a typical extractant for analysis of soluble organic C. In their pioneering work on microbial biomass methodology, Jenkinson and Powlson (1976) also used 0.5 *M* K_2SO_4 to extract C from soil following chloroform fumigation. Brookes et al. (1985) used 0.5 *M* K_2SO_4 as a rapid extraction method for soil microbial biomass N, expecting that K^+ salt was needed to displace NH_4^+ from negatively charged soil surfaces. Potential loss of protein-N from 0.5 *M* K_2SO_4 extracts has not been investigated to our knowledge. Vance et al. (1987a) later used 0.5 *M* K_2SO_4 as a quick extractant for both soil microbial biomass C and N. However, differences in chemical behavior in soil between NH_4^+ and soluble organic C suggest that the same extractant may not be appropriate. Solubilized organic C from microbial origin will likely form associations with various organic constituents (polysaccharides, proteins, etc.) as well as clay fractions, which are then susceptible to "salting out" when extracted with concentrated electrolytic solutions such as 0.5 *M* K_2SO_4 (Theng et al. 1968). Salting out would not be a problem if a consistent fraction of microbially derived C were partitioned, but K^+ can have either a flocculating or dispersing effect on soil solutions, depending upon the dominance of other cations on the soil exchange complex, such as Ca^{2+} or Mg^{2+}. Clayey soils would theoretically be more affected by this "salting out" effect than sandy soils.

Potential problems with using 0.5 *M* K_2SO_4 as an extractant for soluble organic C suggest that other extractants should be investigated. Water is a natural extractant that would avoid addition of electrolytes. In addition, extraction of soluble C with water is often used in microbial ecology studies to investigate the small, but seasonally dynamic component that reflects both substrate for and byproduct of decomposition of organic matter (McGill et al. 1986).

We compared hot and cold water and 0.5 *M* K_2SO_4 as extractants for obtaining estimates of microbially derived C. Our objective was to evaluate if extractant type affected the amount of extractable C in soils that differed in pH.

MATERIALS AND METHODS

Soils were collected at a depth of 0 to 10 cm from eight locations in Oklahoma and Texas during 1997 (Table 1). A single sample from each location was composed of 10 to 20 cores (1.9-cm diameter). Soil pH was determined in 2:1 (water:soil) extracts. Clay content was determined by the hydrometer method (Gee and Bauder 1986). Soil organic C was determined with the modified Mebius method with heating to 150°C for 1 h (Nelson and Sommers 1982).

Experiment 1: Water versus 0.5 *M* K_2SO_4 Extraction

The eight soils were dried at 40°C for 1 d and sieved to pass a 5-mm screen. Subsamples (25 g each) were moistened to 50% field capacity and incubated at 25°C for 7 d to re-establish steady-state microbial activity (Franzluebbers et al. 1996). One subsample was extracted with 100 mL of 0.5 *M* K_2SO_4, shaken for 30 min, filtered through Whatman 42 paper, and analyzed for soluble C with a Model 700 C Analyzer (OI Corp). The same procedure, except with distilled water at room temperature (22°C) and distilled water heated to 100°C as extractants, was repeated on two other subsamples. Two additional subsamples were fumigated with ethanol-free chloroform for 18 h. The extraction procedures with 0.5 *M* K_2SO_4 and water at room temperature were repeated as previously described for unfumigated subsamples. Due to slow filtration of dispersed water extracts, we centrifuged prior to filtration. There was no difference in extractable C between centrifuged and uncentrifuged samples in preliminary tests. The five extraction procedures were replicated three times for each soil.

Experiment 2: Double Extraction with Water, K_2SO_4, and $CaSO_4$

Pullman clay loam (pH 6.5) and Weswood silty clay loam (pH 8.3) soils were sampled again in 1998 and dried at 60°C for 2 d. Six subsamples (25 g each) of each soil were extracted with either (1) water at room temperature (22°C), (2) 0.5 *M* K_2SO_4, or (3) 0.01 *M* $CaSO_4$. Following filtration, the water extract was divided and one half received K_2SO_4 crystals to bring the solution concentration to 0.5 *M* K_2SO_4. Soluble C was analyzed as described previously.

Following filtration, soil retained on filter paper was dried at 60°C and 20 g was re-extracted with either 0.5 *M* K_2SO_4 or water at room temperature for each of the original extractions. For example, the two subsamples originally extracted with water at room temperature were extracted the second time with water at room temperature again or with 0.5 *M* K_2SO_4. The six extraction procedures were replicated three times for each soil.

Both experiments were evaluated using Tukey's Honestly Signficant Difference at $P < 0.05$ in SigmaStat version 2.03 (SPSS Inc. 1997).

Table 1. Characteristics of soils.

Location	Series	Classification	pH	Clay	Organic C
				(mg g^{-1})	
Fay OK	Wheatwood	Fine-silty, mixed, thermic, Fluventic Haplustepts	5.4	150	9.6
Fay OK	Lincoln	Sandy, mixed, thermic Typic Ustifluvents	6.1	70	8.7
Amarillo TX	Pullman	Fine, mixed, thermic, Torrertic Paleustolls	6.7	340	11.4
Thomas OK	Quinlan	Loamy, mixed, thermic, Typic Haplustepts	6.9	260	11.5
Lubbock TX	Acuff	Fine-loamy, mixed, thermic, Aridic Paleustolls	7.4	220	9.3
Corpus Christi TX	Orelia	Fine-loamy, mixed, hyperthermic, Typic Ochraqualfs	7.6	270	11.6
Weslaco TX	Hidalgo	Fine-loamy, mixed, hyperthermic, Typic Calciustolls	8.0	280	9.3
College Station TX	Weswood	Fine-silty, mixed, thermic, Udifluventic Ustochrepts	8.3	310	16.3

RESULTS AND DISCUSSION

Water versus 0.5 *M* K_2SO_4 Extraction

Averaged across soils, water at room temperature extracted more C than did 0.5 *M* K_2SO_4 when unfumigated ($P < 0.001$), but not when fumigated ($P = 0.19$) (Fig. 1). Five of the eight soils had significantly greater extractable C with water compared with 0.5 *M* K_2SO_4 extraction. Differences in extractable C among soils could be explained by differences in soil pH. Extractable C was similar between extractants at high soil pH, but values diverged with decreasing soil pH (Fig. 1).

When unfumigated, soils did not sediment rapidly with water as extractant, but did with 0.5 *M* K_2SO_4 as extractant. Therefore, water acted as a dispersant, keeping soluble C in solution. In contrast, 0.5 *M* K_2SO_4 acted as a flocculant at low soil pH by increasing the electrolyte concentration and reducing the diffuse double layer, which may have allowed solubilized organic C to bind more tightly to clay and organic colloids by intensified electrostatic forces (e.g., Tan 1998). At high soil pH (>7.7), the high concentration of K^+ replaced Ca^{2+} on cation exchange sites. Increased Ca^{2+} in solution increased the diffuse double layer because of the larger outer electron shell of Ca^{2+} than K^+, resulting in a dispersing effect. Potassium sulfate has been used in purifying proteins by "salting out" (Voet and Voet 1990). Potassium sulfate may also affect microbial proteins when fumigated by adsorption of solubilized C to flocculated soil colloids. Some of this solubilized C may be microbial, but not included in the extracts. In both fumigated and unfumigated soils, the difference in extractable C between water and 0.5 *M* K_2SO_4 was unrelated to clay content.

In chloroform-fumigated soils, soil pH did not affect the amount of extractable C in water or in 0.5 *M* K_2SO_4, except in the two soils with pH ≥ 8 where 0.5 *M* K_2SO_4 extracted more C than water (Fig. 1). It is unclear why the same response to soil pH with water as extractant in unfumigated soil did not also appear in fumigated soil. Perhaps chloroform dissolved in soil water acted as a mild flocculant. If this were the case, this flocculating effect may have also been partly responsible for lower extractable C in fumigated than unfumigated soils with low pH. No significant difference in extractable C between water or KCl was also observed in a fumigated soil from California (Kieft et al.

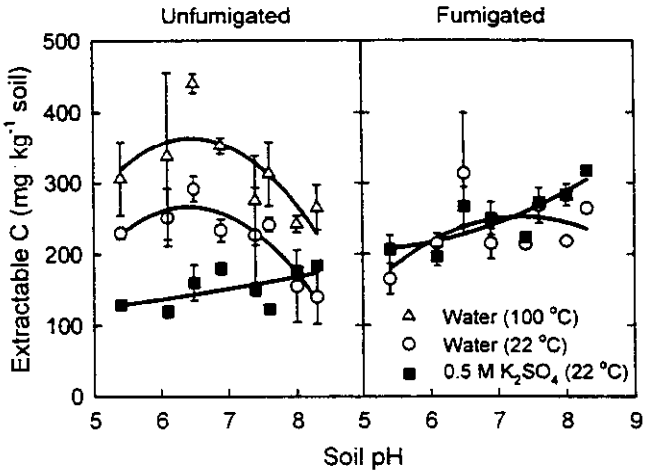

Fig. 1. Extractable C in unfumigated soils with either water at 100°C, water at room temperature, or 0.5 *M* K_2SO_4 and in fumigated soils with either water at room temperature or 0.5 *M* K_2SO_4 as affected by soil pH. Error bars are standard deviation among three replications.

Table 2. Correlation matrix among soil biochemical properties (n = 8 soils)

Property	$K_{F\text{-}C}$	$W_{F\text{-}C}$	$W_{H\text{-}C}$	CFI_F	$CFI_{F\text{-}C}$
$K_{F\text{-}C}$	–	*	NS	NS	*
$W_{F\text{-}C}$	**0.74**	–	NS	†	**
$W_{H\text{-}C}$	0.20	0.39	–	*	NS
CFI_F	0.48	**0.61**	**0.79**	–	†
$CFI_{F\text{-}C}$	**0.80**	**0.85**	0.36	**0.64**	–

$K_{F\text{-}C}$ is traditional chloroform fumigation-extraction with 0.5 M K_2SO_4 (fumigated–control), $W_{F\text{-}C}$ is chloroform fumigation-extraction with water at room temperature (fumigated–control), $W_{H\text{-}C}$ is water at 100°C minus water at room temperature, CFI_F is chloroform fumigation-incubation without subtraction of a control, and $CFI_{F\text{-}C}$ is chloroform fumigation-incubation with subtraction of a 10-d control.

†, *, ** Significant at $P \le 0.1$, $P \le 0.05$, and $P \le 0.01$, respectively; NS, not significant.

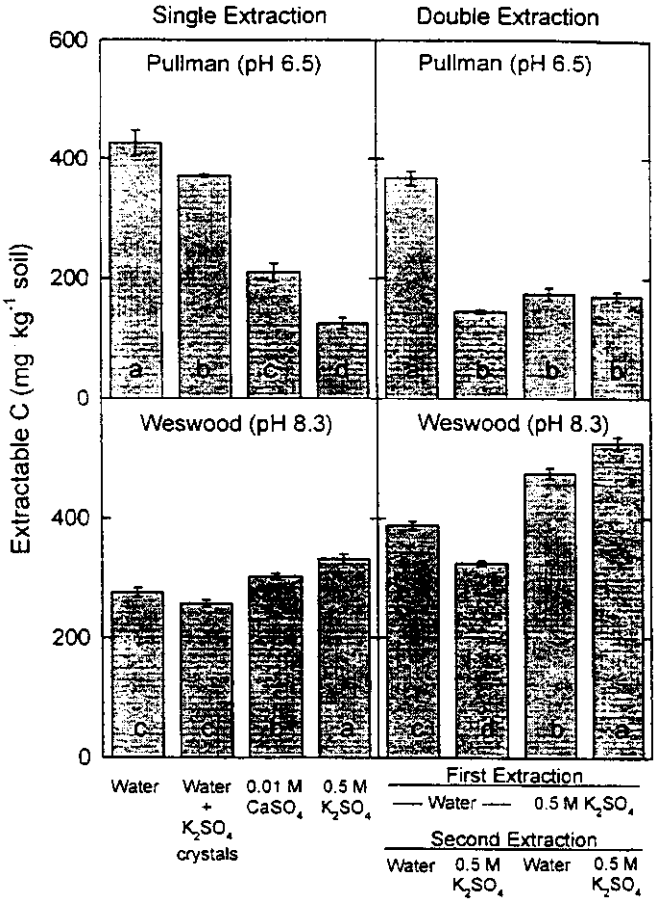

Fig. 2. Extractable C in a low-pH Pullman clay loam and a high-pH Weswood silty clay loam as affected by extractant. Soils initially extracted with either water at room temperature or 0.5 *M* K_2SO_4 were dried at 60°C then extracted again with either water at room temperature or 0.5 *M* K_2SO_4.

1987). Although, like our results, significantly more C was extracted with water than KCl in unfumigated soil.

Modifying the chloroform fumigation-extraction method by using water as extractant yielded negative estimates of microbial biomass C for four of the eight soils, particularly at low pH. Despite negative estimates, chloroform fumigation-extraction with water was correlated with estimates from the traditional chloroform fumigation-extraction with 0.5 *M* K_2SO_4 (Table 2). Extractable C with hot minus cold water was highly related to soil microbial biomass C using the chloroform fumigation-incubation method without subtraction of a control. Further, extractable C with hot minus cold water always yielded positive values. Hot water was used as an alternative to chloroform to kill microbial biomass.

Double Extraction with Water, K_2SO_4, and $CaSO_4$

Water at room temperature extracted 2.9 times more C than 0.5 *M* K_2SO_4 in the low pH soil, but 17% less in the high pH soil (Fig. 2). Reducing the electrolyte concentration of the extractant with 0.01 *M* $CaSO_4$ compared with 0.5 *M* K_2SO_4 resulted in a 44% increase in extractable C in the low pH soil and a 14% decrease in the high pH soil. Addition of K_2SO_4 to bring the water extract to 0.5 *M* resulted in a 14% decline in extractable C in the low pH soil, which further illustrates the effect of high electrolyte concentration of 0.5 *M* K_2SO_4 on possible adsorption of solubilized C to soil colloids <2.5 μm in diameter that passed filtration. These results indicate, though, that the majority (75%) of the difference in extractable C between water and 0.5 *M* K_2SO_4 occurred due to adsorption with particles ≥2.5 μm while in the presence of the entire soil matrix.

When the low pH soil was dried and extracted a second time, extractable C was similar whenever 0.5 *M* K_2SO_4 was used, whether this was K_2SO_4 followed by water, water followed by K_2SO_4, or K_2SO_4 followed by K_2SO_4 (Fig. 2). All extractions that included 0.5 *M* K_2SO_4 resulted in extractable C levels 40 to 47% of those from double water extraction.

In the high pH soil, initial extraction with 0.5 *M* K_2SO_4 resulted in greater extractable C than water followed by water and water followed by 0.5 *M* K_2SO_4. Doubling the electrolyte concentration with repeated extraction using 0.5 *M* K_2SO_4 resulted in a 62% increase in extractable C compared with water followed by 0.5 *M* K_2SO_4.

This experiment illustrated the extreme sensitivity of extractable C to changes in electrolyte concentration that depended upon soil pH. Extractable C decreased dramatically with only small increases in electrolyte concentration in the low pH soil, but increased dramatically only at relatively high K^+ concentrations (i.e., 0.5 *M* K_2SO_4 followed by 0.5 *M* K_2SO_4) in the high pH soil. In the high pH soil, K^+ likely replaced Ca^{2+} on the exchange complex, which caused soils to disperse by increasing the diffuse double layer and allowing solubilized C to stay in solution. Organo-mineral complexes tend to be bound more tightly by Ca^{2+} than by K^+.

The chloroform fumigation-extraction method with 0.5 *M* K_2SO_4 was originally developed for acidic soils that did not perform well using the chloroform fumigation-incubation method with a control subtracted (Vance et al. 1987a,b). Soil microbial biomass C with chloroform fumigation-extraction using 0.5 *M* K_2SO_4 was very similar to that with substrate-induced respiration at low soil pH, but tended to be underestimated at high soil pH (Anderson and Joergensen 1997). Our results, based upon differences in extractable C between water and 0.5 *M* K_2SO_4 in unfumigated soil, but not in fumigated soil, suggest that soil microbial biomass C estimates may be overestimated in soils with pH < 7.7. This pH effect on extractable C agrees with the data comparing chloroform fumigation-extraction and substrate-induced respiration in Anderson and Joergensen (1997). Certainly, further research is needed to better understand the complex biological-chemical-physical interactions that occur in soils upon fumigation and subsequent extraction.

We observed differences in extractable C due to extractant that changed along a soil pH gradient. Our results indicate that solubilized microbial C following chloroform fumigation undergoes various chemical reactions during

extraction that may be dependent upon electrolyte concentration. Adsorption of C compounds to soil colloids and clays appears very likely. Our results put into question the appropriateness of using chloroform fumigation-extraction in a wide range of soils.

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